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PATENT & TRADEMARK OFFICE

Atty. Docket No.: 8822/2022 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Bachmann, et al.	Examiner:	B. Kifle
Serial No.:	10/762,107	Group Art Unit:	1624
Filed:	January 21, 2004		
Entitled:	Farnesyl Dibenzodiazepinone, and Processes for its Production	Conf. No.:	4987

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. JULIAN E. DAVIES UNDER 37 C.F.R. §1.132

I declare:

1. I, Julian E. Davies received a Ph.D. in Organic Chemistry from the University of Nottingham, Nottingham, United Kingdom in 1956. I am currently the Director of the Life Sciences Institute, and Professor Emeritus at The University of British Columbia in the Department of Microbiology and Immunology, of which I served as Department Head from 1992 to 1997.

2. I am not an inventor on the above-noted patent application. My areas of expertise and research include extensive experience in the disciplines of microbiology, virology, parisitology, molecular and cellular biology, genetics, and biochemistry. I have served as President of the American Society of Microbiology, and am currently serving as President of the International Union of Microbiological Societies and as Scientific Director of the Canadian Bacterial Diseases Network. I am an elected Fellow of the Royal Society (UK and Canada), and have been twice awarded the degree of Honorary Doctor of Science (University of Guelph, and University of British Columbia). I presently serve on the editorial boards of Current Opinion in Microbiology (Editor-in-Chief), Research in Microbiology, and Trends in Microbiology, and have served on the editorial boards of Current Opinion in Biotechnology (Editor-in-Chief), The Journal of

Antibiotics, Antimicrobial Agents and Chemotherapy, Journal of Molecular Biology, Annual Reviews of Microbiology, and Journal of Infectious Diseases. I have served at an executive level in 4 different pharmaceutical/biopharmaceutical corporations, and I have sat or am presently sitting on the Scientific Advisory Boards and Review Boards of 5 separate pharmaceutical/biopharmaceutical corporations. I have served, or presently serve as a consultant to 3 different pharmaceutical/biopharmaceutical corporations. A copy of my *curriculum vitae* is attached.

3. I am an inventor on 4 issued patents (3 of which are United States patents). My research has been published in over 225 peer-reviewed articles, and I have authored or co-authored 84 book chapters, symposium proceedings, or commentaries. I am the co-Editor-in-Chief of the *Manual of Industrial Microbiology and Biotechnology* (2^{nd} ed).

4. I have agreed to provide this Declaration, and in this regard I will be receiving remuneration from Ecopia BioSciences Inc. for my services. I have never previously performed paid consulting work for Ecopia Biosciences, Inc.

5. I have read the above-referenced patent application (USSN 10/762,107), and the Office Action issued April 26, 2006 in the above-noted patent application, and I understand that the Examiner has rejected claims 1, 2 and 20-25 for alleged lack of novelty with regard to the disclosure in U.S. Patent No. 5,541,181, issued to Ohkuma et al. ("Ohkuma et al."), in view of the later-published paper by Igarashi et al. (2005), J. Antibiotics 58(5): 350-352. More specifically, the Examiner stated the following at page 2 of the Office Action:

> The claims read on the compound made by the microorganism, strain M990-6, identified as being a species of *Micromonospora*. The reference depicts the structure of the compound isolated incorrectly. However, the later published correction by Igarashi et al. (in J. Antibiot. 58 (5): 350-352 (2005)) revised the structure of the reference.
>
> This anticipation rejection is made on the compound produced by the same microorganism and has the same NMR spectrum as in the prior art (with the difference of solvent peaks). Therefore, the compound claimed was first produced by Ohkuma et al. and the claims read thereon.

6. I have carefully read the Ohkuma et al. patent and the above-referenced Igarashi et al.

(2005) Journal of Antibiotics paper entitled "Revision of the Structure Assigned to the Antibiotic BU-4664L from *Micromonopora* [*sic*]," which were cited in the Office Action.

7. Based on my analyses of the Ohkuma et al. patent and the Igarashi et al. paper, I **cannot agree** with the conclusion that the microorganism used by Ohkuma et al. is the same microorganism used by Igarashi et al., and as well, given my review of the Ecopia patent application (USSN 10/762,107) that the microorganism disclosed therein (*Micromonospora* sp. strain 046-ECO11) is the same microorganism as either of those described in the Okhuma et al. patent or the Igarashi et al. reference. Furthermore, in reviewing both the Okhuma et al. patent and the Igarashi et al. reference, I can find no evidence (*i.e.* scientific data) that would allow for even an expectation on the part of a person of skill in art that, on the basis of the microorganisms isolated by both Okhuma et al. and Igarashi et al. belonging to the genus *Micromonospora*, the microorganisms would produce the same secondary metabolite, namely the compound obtained by Igarashi et al. from their *Micromonospora* sp. strain TP-A-0860. As such, I cannot agree with the conclusion that the compound ECO-4601 claimed in USSN 10/762,107 *was* first produced by Okhuma et al., even though the Okhuma et al. patent clearly does not depict the structure of ECO-4601 nor even imply that such a molecule would have been produced by the *Micromonospora* sp. strain used by Okhuma et al. My explanation for my opinion is set out in the facts presented below.

8. It is known, and accepted as fact, by persons of skill in the art of microbiological sciences and genetics that the actinomycete bacteria comprise a vast number of genetically (and hence physiologically and biochemically) distinct microorganisms that differ in their capabilities to produce secondary metabolite compounds, and that the *Micromonosporaceae* do not form an exception to this rule. It is accepted as fact by those of skill in the art that microorganisms belonging to the genus *Micromonospora* form hundreds of named species (*i.e.* those which have been given a Latin binomial name), while there presently exist thousands of unnamed species of *Micromonospora*. It is accepted as fact by those of skill in the art that any given species of *Micromonospora* may comprise a number of different strains, and that no one strain is indicative of another strain's capabilities to produce any one or more secondary metabolites. That is, it is accepted as fact by those of skill in the art that two different strains of a *Micromonospora* sp. would differ in their genetic makeup, and that there would be **no expectation that the two**

different strains would produce the same secondary metabolites.

9. To properly determine the identity of a *Micromonospora* species strain, a number of criteria require evaluation including cultural (hyphal/spore production and pigmentation) and physiological characteristics, and more definitively, analysis of cell chemistry (fatty acid methyl ester (FAME) analysis) and/or nucleic acid (16S rRNA) composition of the isolated microorganisms.

10. Okhuma et al., in their US Patent 5,541,181, describe the isolation and taxonomic characterization of a strain of *Micromonospora,* which they name as M990-6, and which they isolated from a soil sample collected in Colombo, Sri Lanka. Details of the taxonomic investigations performed by the Okhuma et al. researchers are presented in the Okhuma et al. patent at Example 1 (beginning at column 6, line 7) and these investigations include the results of cellular fatty acid analysis. Production of a farnesylated dibenzodiazepinone compound, namely BU-4664L, by strain M990-6 is described together with a structural determination of the molecule.

11. Igarashi et al., in their paper entitled "Revision of the Structure Assigned to the Antibiotic BU-4664L from *Micromonopora* [*sic*]" published in the *Journal of Antibiotics*, volume 58(5), pages 350-352, describe the isolation and characterization of a farnesylated dibenzodiazepinone compound (*i.e.* a microbial secondary metabolite) from a "rare actinomycete", more particularly from a strain of *Micromonospora* sp. which they have named TP-A0860, and which they isolated from a soil sample collected in Osawano, Toyama, Japan. I have reviewed the Igarashi et al. reference and nowhere within that reference do observe the details of any experimental protocol or results (*i.e.* scientific data, including colony morphology data) from a taxonomic study performed by these authors in relation to their microorganism strain TP-A0860.

Available data demonstrate that M990-6 and 046-ECO11 are different microorganisms:

12. The fact that strains of *Micromonospora* sp. represent distinguishable microorganisms can be illustrated by comparing the fatty acid methyl ester (FAME) profile between two (or more) strains. FAME analysis is a technique accepted by those of skill in the art for comparing strains of microorganisms from even within a single species and differentiating one strain from

another. I have performed such a comparative analysis between the *Micromonospora* sp. strain M990-6 (using the data presented in Table 4 of United States Patent 5,541,181) and *Micromonospora* sp. strain 046-ECO11 (using the data presented in Figure 17 of USSN 10/762,107). The results of my comparison are provided in the Table below:

Fatty Acid Chain (Carbon Atoms in Chain)	***Micromonospora* sp. strain M990-6** (Table 4 of US Patent 5,541,181)	***Micromonospora* sp. strain 046-ECO11** (Figure 17 of US Patent Application No. 10/762,107)
Saturated/Straight	**% of Total Fatty Acids**	**% of Total Fatty Acids**
15	1 %	None detected
16	1 %	4 %
17	6 %	None detected
18	2 %	None detected
19	1 %	None detected
Saturated/Branched		
15:0 iso	20 %	27 %
15:0 ante-iso	5 %	6 %
16:0 iso	8 %	4 %
16:0 9 methyl	None detected	17%
17:0 iso	13 %	14 %
17:0 ante-iso *	25 %	None detected
17:0 iso *	None detected	17 %
Unsaturated		
10 Me16 **	1 %	None detected
(9?) Me16 **	None detected	3 %

17:1 iso	7 %	4 %
18:1 iso	4 %	4 %
10Me17	3 %	None detected
10Me18	1 %	None detected

I note that for fatty acid chains marked with either a single (*) or double (**) asterisk, those chains marked with the same number of asterisks may possibly be considered the same, but have been described with nomenclature that differs between US 5,541,181 and USSN 10/762,107. With regard to the above-noted Table, I observe the presence of significant differences between the *Micromonospora* sp. strain M990-6 and *Micromonospora* sp. strain 046ECO11 with respect to each strain's percentage fatty acid content for all three categories of fatty acid chains: saturated straight chains, saturated branched chains, or unsaturated chains (even if allowance is made for nomenclature differences). On the basis of my comparative analysis presented above, I conclude that given the differences in the relative percentages of the various fatty acids between *Micromonospora* sp. strain M990-6 and *Micromonospora* sp. strain 046ECO11, **these organisms must be regarded as being different microorganisms.**

Strains of the same species are not expected to produce the same secondary metabolites:

13. It is accepted, as fact, by those of skill in the art that even at the same species level, different strains of a *Micromonospora* species are known to make different secondary metabolite antibiotics. For example, the juvenimicins (16-membered macrolides), macquarimicins (tri-cyclic polyketides), neorustimicins (14- membered macrolides) and tetrocarcins (polycyclic glycosides) are each made by different strains of *Micromonospora chalcea.* As such, the fact that two or more strains of microorganism are identical at the genus level, in the present situation belonging to *Micromonospora*, provides no evidence as to the identity of their metabolites, and there is no expectation that such strains would produce the same secondary metabolite.

14. In summary, in my expert opinion, I cannot agree with the statement that the microorganism described in the Okhuma et al. patent as *Micromonospora* sp. strain M990-6 is the same microorganism as that described in the Igarashi et al. reference, namely

Micromonospora sp. strain TP-A0860, nor can I agree that the microorganism described in the Ecopia patent application USSN 10/762,107, namely *Micromonospora* sp. strain 046-ECO11, is (or may be) the same microorganism as that of strain M990-6 or TP-A0860.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

21st June 2006

Date

Julian E. Davies

CURRICULUM VITAE

JULIAN EDMUND DAVIES
Professor Emeritus, Department of Microbiology and Immunology, University of British Columbia

Post-secondary education
1950-1953 Nottingham University - B.Sc. Chemistry, Physics, Math
1953-1956 Nottingham University - Ph.D. Organic Chemistry
1956-1957 Columbia University, New York - Postdoctoral research
1958-1959 University of Wisconsin, Madison - Postdoctoral research

Employment record
1959-1962 Lecturer in Organic Chemistry, Manchester College of Science & Technology
1962-1967 Associate, Dept of Bacteriology & Immunology, Harvard Medical School
1965-1967 Visiting Scientist, Microbial Genetics, Institut Pasteur, Paris, France
1967-1970 Associate Professor, Dept of Biochemistry, University of Wisconsin
1970-1980 Professor, Dept of Biochemistry, University of Wisconsin
1980-1983 Research Director, Biogen S.A., Geneva, Switzerland
1983-1985 President, Biogen S.A, Geneva Switzerland
1985-1991 Chef de l'Unité de Génie Microbiologique, Institut Pasteur, Paris, France
1992-1997 Professor and Head, Dept of Microbiology & Immunology, University of British Columbia
1997–date Professor Emeritus, Dept of Microbiology & Immunology, University of British Columbia
1994-1996 Director, West-East Centre for Microbial Diversity, Vancouver, B.C., Canada
1996-2000 President and CEO, TerraGen Diversity, Inc., Vancouver, B.C.
2000–2004 Executive Vice-President, Technology Development, Cubist Pharmaceuticals, Vancouver, B.C.
2005-2006 Director, Life Sciences Institute, University of British Columbia, Vancouver, B.C.

Current research interests
Origin and evolution of antibiotic resistance genes, especially integron-related. Antibiotic biosynthesis and its regulation. Sub-inhibitory activities of antibiotics, such as cell signaling. Microbial diversity and its applications, especially bioremediation and biotransformation of xenobiotics. Physiology and pathogenicity of gram-positive bacteria such as *S. aureus* and *E. faecalis*. Isolation of antibiotic-producing organisms from commensal populations.

Major awards and distinctions
Fellow of The Royal Society, London 1994; Leeuwenhoek Lecturer 1996
Fellow of The Royal Society of Canada 1996
Fellow of the American Academy of Microbiology 1995
Hoechst-Roussel Award, American Society for Microbiology 1986
Miller Visiting Professor, University of California, Berkeley 1989
Honorary Doctorate of Medicine, University of Zaragoza 1990
Thom Award, Society for Industrial Microbiology 1993
Honorary Doctorate, University of Guelph, Canada 1995
Microbial Chemistry Medal, Kitasato Institute 1991
Scheele Award, Swedish Academy of Pharmaceutical Sciences 1997
Bristol-Myers Squibb Distinguished Achievement Award in Infectious Disease Research 1999
Honorary Doctorate, University of British Columbia 2003

Recent service to science
American Society for Microbiology, President 1999-2000; American Academy of Microbiology Board of Governors 1998-2003; Genome BC Board of Directors 2000-2003; Canadian Bacterial Diseases Network, Scientific Director 2002-2005; International Union of Microbiological Societies, President 2003-2005.
Editorial Boards: Journal of Antibiotics, Current Opinion in Biotechnology, Current Opinion in Microbiology, Annual Review of Microbiology, Research in Microbiology, Trends in Microbial Sciences, Faculty of 1000.
Conferences Organized: NATO Microbial Breeding Course 1987; Spetsai Summer School on Molecular and Cell Biology 1994, 1998, 2002, 2006; Action TB Meeting 1997; International Symposium on the Biology of the Actinomycetes 2001; ASM Northwest Regional Meeting 2003.

Refereed Publications

1. J. Davies, F.E. King and J.C. Roberts. The structure of flaviolin. Chemistry and Industry, 1110-1111 (1954)
2. J. Davies, F.E. King and J.C. Roberts. Studies in mycological chemistry, part II. Proof of the constitution of flaviolin by a synthesis of tri-O-methyl flaviolin. J. Chem. Soc. 2782-2786 (1955)
3. J. Davies, J.C. Roberts and S.C. Wallwork. Sterigmatocystin, a metabolic product of *Aspergillus versicolor.* Chem. and Ind., 178 (1956)
4. J. Davies and J.C. Roberts. Studies in mycological chemistry, part V. Synthesis of 2:5-dihydroxy-7-methyl-1:4-napthaquinone. J. Chem. Soc., 2173-2176 (1956)
5. G. Stork, J. Davies and A. Meisels. The total synthesis of a naturally occurring pentacyclic triterpene system. J. Am. Chem. Soc. **81**:5516 (1959)
6. J. Davies, D. Kirkaldy and J.C. Roberts. Studies in mycological chemistry, part VII. Sterigmatocystin, a metabolite of *Aspergillus versicolor* (Vuillemin) Tiraboschi. J. Chem. Soc., 2169-2178 (1960)
7. G. Stork, A. Meisels and J. Davies. Total synthesis of polycyclic triterpenes: the total synthesis of (+)-alpha-onocerin. J. Am. Chem. Soc. **85**:3419-3425 (1963)
8. J. Davies. Studies on the ribosomes of streptomycin-sensitive and resistant strains of *Escherichia coli.* Proc. Natl. Acad. Sci. **51**:659-664 (1964)
9. J. Davies, W. Gilbert and L. Gorini. Streptomycin, suppression, and the code. Proc. Natl. Acad. Sci. **51**:883-890 (1964)
10. J. Davies, L. Gorini and B.D. Davis. Misreading of RNA codewords induced by aminoglycoside antibiotics. Mol. Pharmacol. **1**:93-106 (1965)
11. J. Davies, P. Anderson and B.D. Davis. Inhibition of protein synthesis by spectinomycin. Science **149**:1096-1098 (1965)
12. J. Davies. Effects of streptomycin and related antibiotics on protein synthesis. Antimicrobial Agents and Chemotherapy 1001-1005 (1965)
13. J. Davies, D.S. Jones and H.G. Khorana. A further study of misreading of codons induced by streptomycin and neomycin using ribopolynucleotides containing two nucleotides in alternating sequence as templates. J. Mol. Biol. **18**:48-57 (1966)
14. J. Davies. Streptomycin and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31**:665-670 (1966)
15. P. Anderson, J. Davies and B.D. Davis. Effect of spectinomycin on polypeptide synthesis in extracts of *Escherichia coli.* J. Mol. Biol. **29**:203-215 (1967)
16. J. Davies. Structure-activity relationships among the aminoglycoside antibiotics. Antimicrobial Agents and Chemotherapy 297-303 (1967).
17. J. Davies and B.D. Davis. Misreading of ribonucleic acid code words induced by aminoglycoside antibiotics: the effect of drug concentration. J. Biol. Chem. **243**:3312-3316 (1968)
18. T. Yamada, D. Tipper and J. Davies. Enzymatic inactivation of streptomycin by R factor-resistant *Escherichia coli.* Nature **219**:288-291 (1968)
19. J. Davies and F. Jacob. Genetic mapping of the regulator and operator genes of the *lac* operon. J. Mol. Biol. **36**:413-417 (1968)
20. B. Weisblum and J. Davies. Antibiotic inhibitors of the bacterial ribosome. Bacteriological Reviews **32**:493-528 (1968)
21. J. Davies, R. Benveniste, K. Kvitek, B. Ozanne and T. Yamada. Aminoglycosides: biologic effects of molecular manipulation. J. Infect. Dis. **119**:351-354 (1969)
22. A. Bollen, J. Davies, M. Ozaki and S. Mizushima. Ribosomal protein conferring sensitivity to the antibiotic spectinomycin in *Escherichia coli.* Science **165**:85-86 (1969)
23. B. Ozanne, R. Benveniste, D. Tipper and J. Davies. Aminoglycoside antibiotics: inactivation by phosphorylation in *Escherichia coli* carrying R factors. J. Bacteriol. **100**:1144-1146 (1969)
24. A. Bollen, T. Helser, T. Yamada and J. Davies. Altered ribosomes in antibiotic-resistant mutants of *E coli.* Cold Spring Harbor Symp. Quant. Biol. **34**:95-100 (1969)
25. R. Benveniste, T. Yamada and J. Davies. Enzymatic adenylylation of streptomycin and spectinomycin by R-factor resistant *Escherichia coli.* Infect. and Immun. **1**:109-119 (1970)
26. J. Davies. Structure-activity relationships among the aminoglycoside antibiotics: comparison of the neomycins and hybrimycins. Biochim. Biophys. Acta **222**:674-676 (1970)

27. J. Davies, M. Brzezinska and R. Benveniste. R-factors: biochemical mechanisms of resistance to aminoglycoside antibiotics. Ann. N.Y. Acad. Sci. **182**:226-233 (1971)
28. R. Benveniste and J. Davies. Enzymatic acetylation of aminoglycoside antibiotics by *Escherichia coli* carrying an R-factor. Biochemistry **10**:1787-1796 (1971)
29. T. Yamada and J. Davies. A genetic and biochemical study of streptomycin and spectinomycin-resistance in *Salmonella typhimurium.* Molec. Gen. Genetics **110**:197-210 (1971)
30. J. Davies. Bacterial resistance to aminoglycoside antibiotics. J. Infect. Dis. **124**(Suppl):s7-s10 (1971)
31. R. Benveniste and J. Davies. R-factor mediated gentamicin resistance: a new enzyme which modifies aminoglycoside antibiotics. FEBS Lett. **14**:293-296 (1971)
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33. R. Littlewood, B. Shaffer and J. Davies. Mutants of *S. Cerevisiae* with reduced levels of ribonuclease activity. Genetics **68**(Suppl.):s39 (1971)
34. T.L. Helser, J. Davies and J.E. Dahlberg. Mechanism of kasugamycin resistance in *Escherichia coli.* Nature New Biology **235**:6-9 (1972)
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36. J. Davies. Bacterial resistance to antimicrobial agents. Triangle **11**:131-135 (1972)
37. J. Davies. Mechanisms of resistance to antibiotics. Ann. Reports Medicinal Chemistry **7**:217-227 (1972)
38. J. Davies and M. Nomura. Genetics of bacterial ribosomes. Ann. Rev. Genetics **6**:203-234 (1972)
39. J. Davies and R. Rownd. Transmissible multiple drug resistance in enterobacteriaceae. Science **176**:758-768 (1972)
40. R. Benveniste and J. Davies. Mechanisms of antibiotic resistance in bacteria. Ann. Rev. Biochemistry **42**:471-506 (1973)
41. B.S. Littlewood and J.E. Davies. Enrichment for temperature-sensitive and auxotrophic mutants in *Saccharomyces cerevisiae* by tritium suicide. Mutation Research **17**:315-322, (1973)
42. M. Brzezinska and J. Davies. Two enzymes which phosphorylate neomycin and kanamycin in *Escherichia coli* strains carrying R factors. Antimicrob. Agents Chemother. **3**:266-269 (1973)
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44. M. Cannon, J. Davies and A. Jimenez. Inhibition by lomofungin of nucleic acid and protein synthesis in *Saccharomyces cerevisiae*. FEBS Lett. **32**:277-280 (1973)
45. R. Benveniste and J. Davies. Aminoglycoside antibiotic-inactivating enzymes in actinomycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. Proc. Natl. Acad. Sci. **70**:2276-2280 (1973)
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54. D.E. Berg, J. Davies, B. Allet and J.-D. Rochaix. Transposition of R factor genes to bacteriophage lambda. Proc. Natl. Acad. Sci. **72**:3628-3632 (1975)

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61. E. Cundliffe and J. Davies. Inhibition of initiation, elongation, and termination of eukaryotic protein synthesis by trichothecene fungal toxins. Antimicrob. Agents Chemother. **11**:491-499 (1977)
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